The effect of high dose morphine compared to fentanyl infusion on serum levels of mast cell tryptase during cardiac surgery

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Declaration

I hereby declare that the content of this thesis is my own original work and that it has not previously been used in whole or in part in obtaining another degree or diploma.

Signed: .....

Date: 7 January 2016

Opsomming

Agtergrond: Morfien aktiveer kutane mastselle direk op 'n oënskynlik dosis-afhanklike wyse, wat lei tot die vrystelling van beide histamien en tryptase in die bloedstroom. Tryptase word feitlik uitsluitlik gestoor in mastselle. Verhoogde serum tryptase konsentrasie dien as 'n aanduiding van mastsel aktivering en het die mees gebruikte laboratorium ondersoek in anafilakse geword. Na 'n kliniese diagnostiese dilemma, is ons studie daarop gemik om die vraag te beantwoord of mastsel tryptase konsentrasie steeds nuttig is in die diagnose van anafilakse selfs na toediening van hoë dosis morfien.

Metodes: Ons het 'n nie-geblinde, gerandomiseerde studie gedoen om die effek van fentaniel en hoë dosis morfien op serum massel tryptase konsentrasies te vergelyk. 'n Krag analise is uitgevoer. Twintig volwassenes wat hartchirurgie sou ondergaan is ewekansig toegewys aan een van twee opioïed regimens. Beide groepe het 'n fentaniel bolus van tussen 3 en 8 mkg/kg met induksie ontvang. In die fentaniel groep is dit gevolg deur 'n fentaniel infusie van 5 tot 10 mkg/kg/uur tot die einde van chirurgie. Pasiënte in die morfien groep het morfien 1 mg / kg oor dertig minute ontvang. Serum mastsel tryptase konsentrasies is bepaal direk voor induksie van narkose en weer 90 minute na die aanvang van die opioïed infusie. Die primêre eindpunt was statistiese verskille in tryptase konsentrasie tussen die morfien en fentaniel groepe by die twee tydpunte.

Resultate: Tien pasiënte met soortgelyke demografie is ingeskryf in elke groep. In die fentaniel groep was die tweede, 90 minute mastsel tryptase konsentrasie statisties beduidend (10.1%) laer (P = 0,006) as basislyn. Ten spyte daarvan dat die 95% -vertrouensintervalle van die verskil tussen die gemiddelde (-1,06 tot -0,34 mkg/L) nie nul insluit nie, sluit dit nie 'n klinies belangrike verskil in nie. In die morfien groep het serum mastsel tryptase konsentrasies in die tweede (90 minute) monster nie statisties betekenisvol verskil van die basislyn waardes nie, en sluit die 95% -vertrouensintervalle nul in. Geen tussen-groep verskille in tryptase konsentrasie is opgespoor nie. Een pasiënt in die morfien groep het 'n klinies beduidende toename van 50,4% in tryptase

konsentrasies gehad, hoewel vanaf 'n hoë basislyn van 11,9 mkg/L. In hierdie klein studie is dit 'n voorkoms van 10% (95% CI 1.8% tot 40.4.)

Gevolgtrekking: In hierdie ~~l~~oodsstudie is serum mastsel tryptase konsentrasies nie beinvloed deur of fentaniel of hoë dosis morfien toediening nie. Die nul-hipotese, dat daar geen beduidende toename in serum mastsel konsentrasie na hoë dosis morfien in vergelyking met fentaniel tydens kardiale narkose en chirurgie is nie, is dus aanvaar. Groter studies word egter benodig om 'n seker gevolgtrekking te kan maak, veral in die morfien groep.

Summary

Background: Morphine directly activates cutaneous mast cells in a seemingly dose-dependent manner, resulting in the release of both histamine and tryptase into the bloodstream. Tryptase is almost exclusively stored in mast cells. Elevated serum tryptase concentrations serve as an indicator of mast cell activation and have become the most frequently used laboratory investigation in anaphylaxis. Following a clinical diagnostic dilemma our study was aimed at answering whether mast cell tryptase concentration remains useful in supporting the diagnosis of anaphylaxis even after administration of high dose morphine.

Methods: We conducted a non-blinded, randomized controlled trial comparing the effects of fentanyl and high dose morphine, on serum mast cell tryptase concentrations. A power analysis was performed. Twenty adults undergoing cardiac surgery were randomly assigned to one of two opioid regimens. Both groups received a fentanyl bolus of 3 to 8 mcg/kg at induction. In the fentanyl group this was followed by a fentanyl infusion of 5 to 10 mcg/kg/hr until completion of surgery. Patients in the morphine group received morphine 1 mg/kg infused over thirty minutes. Baseline serum mast cell tryptase concentrations were determined directly prior to induction of anaesthesia and again 90 minutes after the start of the opioid infusion. The primary endpoint was statistical differences in tryptase concentrations between the morphine and fentanyl groups at the two time periods.

Results: Ten patients of similar demographics were enrolled in each group. In the fentanyl group the second, 90-minute mast cell tryptase concentration was statistically significantly (10.1%) lower ($p = 0.006$) than baseline. Despite the 95% confidence interval of the difference between the means (-1.06 to -0.34 mcg/L) not including zero , this was not a clinically important difference.

In the morphine group serum mast cell tryptase concentrations in the second (90 minute) sample were not statistically different from baseline values, the 95% confidence interval including zero. No between-group differences in tryptase concentration were detected. One patient in the morphine group exhibited a clinically significant 50,4% increase in tryptase concentrations, albeit from a high baseline of 11.9 mcg/L, which in this small study constitutes a prevalence of 10% (95% CI 1.8% to 40.4.)

Conclusion: In this small pilot study, serum mast cell tryptase concentrations were unaffected by whether fentanyl or high dose morphine was administered. The null hypothesis, that there is no significant increase in serum mast cell concentrations after high dose morphine compared to fentanyl during cardiac anaesthesia and surgery, was therefore accepted. Larger studies are however needed to ensure a robust result, especially in the morphine group.

Table of Contents

Table of Contents

Detailed literature review

Mast cell physiology

Introduction

Mast cells were first described by Paul Ehrlich during the 18th century.[1,2] They are immune cells that play a crucial role in the initiation of allergic diseases and type 1-hypersensitivity reactions like anaphylaxis.[3,4] Other functions are varied and may include involvement in inherent and adaptive immunity,[2] some autoimmune diseases,[5] tissue repair[4] as well as protection from certain bacterial and parasitic infections.[1,6]

Mast cell morphology

Mast cells are normally oval to stretched-out in shape with a single non-segmented nucleus.[7] The cytoplasm of mast cells is tightly packed with granules containing histamine, proteases (enzymes which break down proteins and peptides) and proteoglycans like heparin.[8] The surface of mast cells contain high affinity immunoglobulin E (Fc epsilon) receptors, low-affinity immunoglobulin G receptors, assorted G-protein coupled receptors, Toll-like receptors,[9] as well as receptors for stem cell factor.[2,7,10]

Mast cell development

Mast cells stem from CD34+ multipotent hematopoietic cells present in bone marrow.[2,4,11] Unlike basophils, mast cells are released into the circulation as immature cells and only undergo maturation and further development when they reach their specific target tissues.[2,3,12] Although found scattered throughout skin and mucosa, they are especially plentiful in areas close to blood vessels and in surfaces that lie close to the outside environment.[13]

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Mast cell classification

Mast cells are categorized into different subtypes according to the kind of protease enzymes stored in their cytoplasmic organelles[8,11] and consist of:

1. Mast cells containing **tryptase** stored in their granules: this type is most predominant in lung tissue,[11]
2. Mast cells containing **chymase and tryptase**: this type is concentrated in skin,[11,13] and
3. Mast cells containing only **chymase** without tryptase in their granules. [11]

Mast cell heterogeneity

An important concept in mast cell biology is that mast cells "do not represent a homogenous population".[2] Not only do they store different biologically active substances in their cytoplasmic organelles as outlined above, they also differ with regard to:

1. The locations and conditions in which they mature (their "microenvironments"),[2] and
2. Their vulnerability to activation and subsequent response to stimulation.[4,6]

Just as mast cells in different locations display distinct characteristics, similarly mast cells found in the same areas or tissues may be significantly different from each other.[2]

Mast cell activation mechanisms

The activation mechanism of mast cell can be considered to be mediated through either:

1. Binding at the high-affinity immunoglobulin E receptor (FcεRI), and/or
2. Mechanisms that do not involve the immunoglobulin E receptor.[2]

The most frequent mechanism of mast cell activation is via antigen binding to the FcεRI receptor.[14] Mast cell activation involves degranulation followed by synthesis of new mediators, predominantly chemokines, cytokines, and arachadonic acid metabolites. The signalling pathways initiating eicosanoid and cytokine production are not well understood and appear to be distinct from those involved in degranulation.[15]

The process of IgE mediated degranulation occurs is relatively well understood. Previous contact with a foreign antigen results in the production of specific IgE antibodies. These antibodies become bound to IgE receptors on mast cells.[11] When the same antigen is again introduced it binds to immunoglobulin molecules on the mast cell surface ("cross-linking of the Fc-bound IgE").[2] These bound receptors come together in specialized areas on the mast cell membrane to initiate the activation of mast cells.[15] The exact mechanism involved in the intracellular signalling processes that follow after the convergence of receptors are still not completely understood.[2,15] Two second-messenger systems involved in the signalling process are inositol trisphosphate (IP_3) and diacylglycerol (DAG). Receptor binding activates the enzyme phospholipase C gamma, an isoform of phospholipase C; this ultimately culminates in the production of IP_3 and DAG.[15] IP_3 releases calcium stored in the endoplasmic reticulum and DAG activates the enzyme protein kinase C. These two events act together to trigger exocytosis.[2,15]

Mast cells can be activated by a variety of mechanism that do not involve the high-affinity immunoglobulin E receptor, the most important of these being:

1. Complement (Anaphylatoxins): C3a and C5a activate specific receptors for complement fragments,[11]
2. Bridging of Immunoglobulin G (IgG) Receptors,[2,7]
3. Stem cell factor through its receptor c-KIT,[15]
4. Neuropeptides and cytokines like substance P, vasoactive intestinal peptide along with some interleukins,[2]
5. Activation of Toll-like receptors on the mast cell surface (i.e. through the action of bacterial products),[9]
6. Toxins like snake and wasp venom,[10]
7. Drugs including contrast media, curare and opioids like morphine[16] and pethidine, and/or
8. Morphine that appears to degranulate mast cells via a mechanism that involves neither

immunoglobulin receptors nor opioid receptors is discussed later.

Mast cell mediator production

Following mast cell activation, secretion of granular content may occur in various ways, these being anaphylactic degranulation, piecemeal degranulation, or degranulation-independent chemokine secretion.

Anaphylactic degranulation follows IgE mediated mast cell activation and is the best know mechanism of mast cell mediator release. Following activation, stored mediators are released rapidly and completely via exocytosis to mediate immediate hypersensitivity reactions. In this process the membranes of the secretory granules fuse with each other as well as the plasma membrane to expel granular content into the extra-cellular environment.[17-19]

In contrast to anaphylactic degranulation, piecemeal degranulation is a slower process of secretion whereby certain mediators can be released 'selectively' without degranulation taking place. It has been identified as a mechanism of mast cell secretion during many inflammatory diseases such as urticarial pigmentosa, bullous pemphigoid and ulcerative colitis. During piecemeal degranulation the mast cell granules slowly lose their content. Ultra-structural characteristics of piecemeal degranulation include a combination of the following features within the same cell:

1. Normal mast cell granules,
2. Empty granules, and/ or
3. Altered granules.

It has been suggested that mast cell granules empty and fill through the action of small vesicles in the cytoplasm, the so-called "Vesicular shuttling hypothesis". The triggers of selective release is not as well know as those causing anaphylactic degranulation.[17,19,20] Selective release has been demonstrated for substances such as eicosanoids[21] and serotonin.[22]

Degranulation-independent chemokine secretion is another form of mast cell activation. Fischer et al. showed that mast cells could be selectively activated to synthesize chemokine's without the release of pre-formed mediators via degranulation or the production of leukotriene's.[20]

Mast cell mediators and their functions

Mast cell activation, regardless of the underlying mechanism, results in a diverse collection of biologically active substances both secreted and subsequently produced by activated mast cells. These can be divided as follows:

1. **Immediate release of pre-formed granular mediators:** There are substances stored and "pre-packaged" in cytoplasmic granules, ready for rapid release during mast cell degranulation,
2. Synthesis of membrane lipid mediators, e**icosanoids,** occurs within minutes and
3. The synthesis of **cytokines and chemokine's** is delayed by minutes to hours.[7,23,24]

Pre-formed mediators within the cytoplasmic granules of mast cells contain mainly histamine, heparin and proteases. Proteases present in mast cells include tryptase, chymase and carboxypeptidase, tryptase being the most frequently measured of the three.[11,25] In addition, these organelles also store proteoglycans like heparin. It appears that their function is to bind to and stabilize, other substances in the granules.[7]

Histamine is derived from histidine (an amino acid) and is present in both mast cells as well as basophils.[25] The effects are mediated through G-protein coupled receptors, delineated H1 to H4. Stimulation by histamine results in:

1. Vasodilatation and increased vascular permeability via the production of nitric oxide and activation of potassium channels in vascular endothelium,
2. Increased cardiac inotropy and chronotropy and ultimately increased cardiac output,
3. Urticaria and angioedema,
4. Bronchospasm and coronary artery spasm from contraction of smooth muscle,
5. Abdominal pain from contraction of smooth muscle and
6. Increased viscosity of mucous and increased glandular secretions.[2,7,10,25]

Mast cell tryptases are enzymes with trypsin-like characteristics.[5] Together with chymases they are the major proteins in mast cell granules.[10] The major tryptase subtypes are membrane-bound gamma tryptase and soluble tryptases. The latter are divided into alpha, beta and delta gamma tryptases,[7] with Beta-tryptases divided in to beta 1,2,3 and alpha-tryptases are divided into alpha 1 and 2 subgroups. [10,11] Beta tryptase is composed of four subunits (creating a tetramer); each subunit containing one active enzyme site.[26] All the active enzyme sites of the tryptase tetramer face inward towards a central opening. This configuration protects the compound from enzymatic breakdown as protease inhibitors have difficulty accessing their binding sites.[26] Neither the biological role of mast cell tryptase nor its regulation in vivo is completely understood.[26] However the functions of mast cell tryptase are thought to comprise:

1. Stimulation of growth and development of certain cells,[11]
2. Attraction of immune cells to areas of inflammation,[3] and
3. Activation of nearby mast cells in an "autocrine action". [3]

Tryptases exerts their effects through G-protein coupled receptors known as protease activated receptors (PAR's) that are found in, amongst other places, the respiratory tract and the vascular system.[11]

Eicosanoid lipid mediators are synthesised after degranulation occurs. These eicosanoids are produced from twenty-carbon essential fatty acids, usually cell membrane arachidonic acid. This process that requires the enzyme phospholipase A_2.[24,27] The ultimate end products, leukotrienes and prostaglandins, are then produced through the cyclooxygenase and 5-lipoxygenase pathways respectively.[7,25]

Prostaglandin D_2 is the chief prostaglandin produced by mast cells. It acts on prostaglandin receptors present in most tissues to mediate effects such as:

1. Peripheral vasodilatation,
2. Coronary and pulmonary artery constriction, and
3. Bronchospasm.[25]

Prostaglandin F_2 Alpha, Prostaglandin E_2 and thromboxane A_2 are also synthesized, all of which can cause bronchoconstriction and vasodilatation.[25]

Leukotrienes also act through G-Protein coupled receptors to induce:

1. Bronchoconstriction,
2. Increase vascular permeability, and/or
3. Mucous production.[7]

Platelet activating factor is a leukotrine which stimulates platelet aggregation, bronchoconstriction and increases vascular permeability.[25]

Chemokine and cytokine production represents the final consequence of mast cell activation

is the synthesis of various chemokines and cytokines. The production of cytokines and chemokines gives rise to the biphasic phase of anaphylactic reactions.[24] Tumour necrosis factor-alpha is the major cytokine released. TNF-alpha together with interleukins 1 to 6 are responsible for:

1. Chemotaxis,
2. Enhanced bronchial responsiveness, and/or
3. Sensitization to vasoactive mediators.[7,25]

Disorders of mast cell activation and/or proliferation

Mast cells are best known for their role in immediate hypersensitivity reactions like anaphylaxis as well as allergic disorders such as asthma and urticaria. They are also suspected to be involved in other chronic inflammatory disorders such as multiple sclerosis. Diseases arising from abnormal and excessive mast cell production are generally referred to as mastocytosis.

Systemic mastocytosis

Systemic mastocytosis describes a group of disorders that result from the abnormal accumulation of mast cells and their precursors in various tissues.[28-30] Mastocytosis occurs in all age groups and is frequently linked to a mutation in the receptor for stem cell factor (KIT).[10] Symptoms may include headaches, dizziness, flushing, urticaria, hypotension, diarrhoea and abdominal pain.[30,31] The symptoms of mastocytosis arise due to:

1. Uncontrolled mast cell production,
2. Infiltration and accumulation of mast cells within organs (i.e. bone marrow, skin liver, spleen and lymph nodes),[10] and/or
3. Release of mast cell mediators.[30]

The World Health Organization divides mastocytosis into three main categories, these being:

1. Cutaneous mastocytosis (only skin involvement),
2. Systemic mastocytosis (extra-cutaneous involvement) with multiple sub-types, i.e. indolent, aggressive, non-mast cell clonal haematological disease, and
3. Mast cell neoplasms such as mast cell leukaemia. [28,29]

The diagnostic criteria for systemic mastocytosis are listed in Table 1. To establish the diagnosis, either a major criterion together with one minor criterion or all three minor criteria should be present.[28]

Table 1. **Diagnostic WHO criteria for systemic mastocytosis (SM) criteria**
Major Criteria Multifocal compact infiltrates of mast cells in bone marrow or other extra-cutaneous organ(s). (more than 15 mast cells)

Minor Criteria

a. Mast cells in bone marrow or other extra-cutaneous organ(s) show an abnormal spindle shaped deformity (>25%)

b. C-kit mutation D816V in extra-cutaneous organ(s)

c. Serum tryptase >20ng/ml (does not count in patients who have an associated haemopoietic clonal non mast cell lineage disease. (=AHMND)

d. Mast cells in the bone marrow express CD 2 and/or CD 25

If at least one major and one minor criterion or three minor criteria are fulfilled, the diagnosis of systemic mastocytosis can be established.

[1] other activating mutations at codon 816 of c-kit also count as a minor criterion

Mast cell tryptase (total tryptase levels) are used both to diagnose systemic mastocytosis and also to monitor the response to treatment. Total tryptase levels reflect the 'mast cell burden' as mast cells continuously secrete immature pro-alpha tryptase, thus tryptase levels parallel the number of mast cells present, the so called 'mast cell burden'.[30]

Monoclonal mast cell activation syndrome (MMAS)

Patients with monoclonal mast cell activation syndrome have symptoms suggestive of mast cell activation (periodic hypotension, flushing, urticaria etc.) but do not meet the diagnostic criteria for systemic mastocytosis.[32]

Anaphylaxis

The National Institute of Allergy and Infectious Diseases/ Food Allergy and Anaphylaxis Network Symposium **define** anaphylaxis as a "serious allergic reaction that is rapid in onset and may cause death".[33-36] Estimated lifetime prevalence has been quoted to be between 0.05 to 2% with an **incidence** under anaesthesia thought to range between 1 in 10 000 to 1 in 20 000 procedures.[24,27,33,34,37-42] The occurrence of anaphylaxis during anaesthesia seems to be increasing.[24,43]

The pathophysiology of anaphylaxis involves the activation of mast cells and basophils, which can occur through both immune and non-immune mechanisms.[24] Immune-mediated or so-called "allergic anaphylaxis" is most commonly initiated by immunoglobulin E binding to its high affinity receptor on mast cells and basophils.[43] Other non-IgE dependent immune pathways include complement activation through immunoglobulin M, immunoglobulin G, and immune complexes.

Non-Immune mediated anaphylaxis occurs through direct activation of mast cells and basophils.[39,43,44] Triggers for direct mast cell degranulation may include:

1. Drugs such as morphine, possible mechanisms are discussed later,
2. Toxins and chemicals[5] as well as
3. Contrast media and radiation.[38,44,45]

Clinical signs of Anaphylaxis usually start appearing anywhere between five to thirty minutes after contact with an allergen, although it can sometimes be delayed by several hours. Biphasic reactions occur in up to 20% of patients without being re-exposed to the agent. It normally occurs in the first eight hours but has been reported to occur as late as 72 hours after the initial event.[37] Immune-mediated anaphylactic reactions tend to become more severe when the

patient is re-exposed to the same agent. This is unlike non-allergic reactions, which are usually more dependent on the total amount of antigen given.[43] The main organ systems involved in order of frequency are: skin/mucosal, respiratory, gastrointestinal, cardiovascular and neurological.[24,38] Clinical features of anaphylaxis can range from mild, localized skin signs to life-threatening multi-organ failure.[33] Life-threatening features may include laryngeal oedema resulting in upper airway obstruction, severe bronchospasm, and anaphylactic shock with tachycardia, hypotension, bradycardia and ultimately cardiac arrest.[33,34,37,38]

Anaphylaxis is a clinical diagnosis.[27,37] Contact with a known trigger, time to the start of symptoms and progression of signs to involve more than one organ system is very suggestive.[24] Clinical diagnosis is difficult in patients undergoing anaesthesia as patients are covered by surgical drapes, have multiple drug administrations and access to the patient is limited.[46,47] Markers of massive mast cell degranulation such as mast cell tryptase and plasma histamine can be valuable in supporting the diagnosis of anaphylaxis.

Markers of mast cell activation

The most commonly performed laboratory investigations to confirm mast cell activation are plasma histamine and tryptase levels. As both mature beta tryptase and histamine is stored in mast cell organelles and are released during mast cell degranulation, raised levels detected systemically thus point toward mast cell activation.[24] Neither plasma histamine levels nor total tryptase levels are currently optimally sensitive or specific for the diagnosis of anaphylaxis.[24,27]

Histamine and histamine metabolites may be used as markers of mast cell activation. Histamine

is stored in both mast cells and basophils.[36] It is broken down rapidly by the enzyme histamine methyl transferase, which is present in most tissues.[11,25] Due to its rapid breakdown histamine has a short half-life [48]; this means that plasma histamine levels normally peak early at approximately 5-10 minutes and disappear within 30-60 minutes.[11,37,38] Obtaining histamine levels after anaphylaxis is difficult as levels need to be taken within one hour after the start of symptoms (during which time resuscitation is taking place).[27,37] Furthermore, specimens require special handling, and should be:

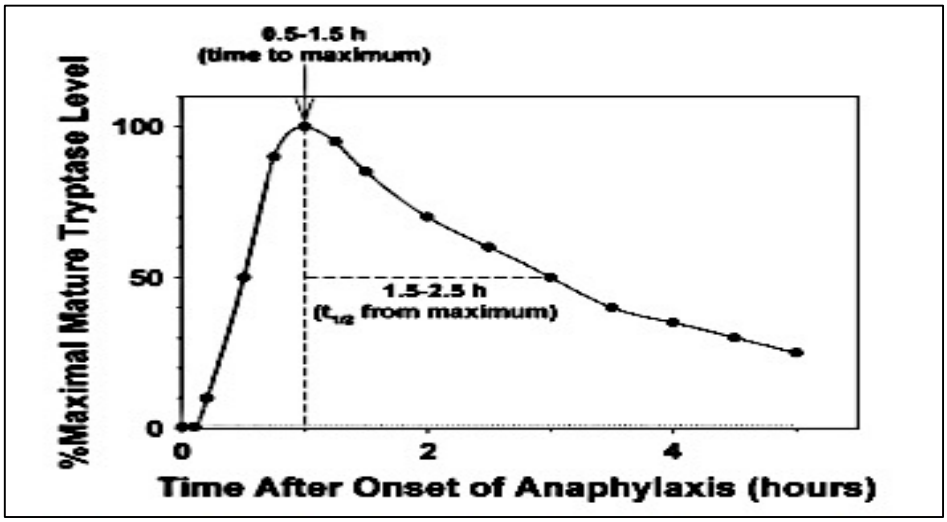
1. Taken using a wide bore needle,
2. Cooled by placing on ice and then
3. Centrifuged and plasma frozen immediately.[24]

An alternative not commonly used in anaesthesia is to measure histamine metabolites in 24-hour urinary samples.[27]

Mast cell tryptase is the most frequently used laboratory test in anaphylaxis as it is considered to be more specific than histamine.[29,30,49,50] Tryptase is found mostly in mast cells, with only trace amounts stored in circulating basophils (basophils contain roughly 0.002% of the tryptase that is present in mast cells)[48] Because tryptase is essentially exclusive to mast cells, elevated levels in serum are used as an indicator of mast cell activation.[5,51,52] Resting mast cells normally secrete immature pro-beta and pro-alpha tryptases, (predominantly pro-beta tryptases) creating a baseline blood level.[26,48,51,53-55] This resting serum level is regarded as an indication of mast cell 'load', being elevated in conditions like mastocytosis, where excessive amounts of mast cells accumulate in tissues.[26] Mast cell granules store just the mature form of beta tryptase, which is released via exocytosis only after mast cell activation. Mature beta tryptase thus denotes mast cell degranulation and should not normally be present in serum.[26]

Beta tryptase within the mast cell granules bind to proteoglycan groups (like heparin), forming large stable compounds.[5] The large size of the tryptase-proteoglycan complex means that after its release diffusion is slow, explaining the later appearance of tryptase in the circulation as compared to histamine whose levels peak early, despite the fact that they are secreted at the same time[26,48] Based on research conducted by Schwartz et al., beta tryptase levels reached maximum values at approximately 1-2 hours after wasp sting venom induced anaphylaxis (Figure 1). The half-life of beta tryptase was found to be between 1.5 hours and 2 hours.[26]

Figure 1.



The hypothetic time course for the appearance of mature beta tryptase in serum or plasma during systemic anaphylaxis. The maximum level is set at 100% in the figure, however in reality, it varies at least in part depending on the severity and nature of the anaphylactic stimulus, which in turn affects how long mature tryptase is in the detectable range.

The longer half-life of mature beta tryptase means that serum levels should ideally be taken within 2 hours after the anaphylactic episode. Serum levels will also stay raised for up to 6 hours, only returning to normal resting levels after 14 hours.[26,48] After a suspected anaphylactic episode it is recommended that a blood sample should be taken an hour after the start of the allergic reaction to determine peak tryptase levels and again after 24 hours to document baseline tryptase levels.[56] There appears to be an association between the severity of the allergic reaction and the peak tryptase levels in some studies.[52] Normal baseline levels of total tryptase lie between 1 to 15 mcg/L, although other, lower reference values exist.[26] The accurateness of mast cell tryptase levels in diagnosing serious allergic reactions can also be improved by:

1. Preforming multiple measurements (determining at least a peak and baseline value),
2. Measuring plasma histamine levels simultaneously, and/or
3. Measuring both mature beta tryptase and total tryptase levels.[56] A ratio of total to mature beta tryptase of 10 or below is indicative of anaphylaxis.[26,57]
   An increase in total tryptase levels of 20% or more from the baseline is suggestive of mast cell activation.[49,58]

Tryptase levels are much more likely to be raised when anaphylaxis is triggered by a parentally administered allergen, when the reaction is immunologically mediated and when the reaction is severe and hypotension results.[24] This is probably due to delivery of large amounts of allergen directly to mast cells resulting in rapid and extensive mast cell degranulation.[26]

Patients with elevated baseline levels of tryptase can have false-positive results when measuring only peak tryptase levels to investigate anaphylaxis. Elevated baseline total tryptase levels may occur in patients with:

1. Mast cell disorders like mastocytosis,
2. Other myelodysplastic and myeloproliferative disorders,
3. Acute or chronic myeloid leukemia,
4. Hypereosinophillic syndrome,
5. Allergic or atopic disorders,
6. Trauma,
7. Myocardial infarction,
8. Heroin use,
9. Hypoxia, and/or
10. End-stage kidney disease.[5,10,26,58]

False-positive tests have also been described in post-mortem samples in patients whom had

coronary artery disease, salicylate poisoning and whom suffered multiple trauma.[26,48,53-55,59] In some cases of anaphylaxis, tryptase levels may not become notably elevated, meaning that the diagnosis of anaphylaxis cannot be excluded by a negative result.[35,36,58,60] False-negative results may be attributed to:

1. Localized mast cell activation, the extent of which is not great enough to increase systemic serum tryptase levels,[5] or
2. Anaphylaxis possibly mediated through basophils, which contain only minute quantities of tryptase as compared to mast cells,[5] or
3. Food-induced anaphylaxis. This may be as a result of:
    3.1. Smaller peak tryptase levels occurring later in the course of the reaction due to the more gradual onset of food induced anaphylaxis,
    3.2. Local activation of mucosal mast cells confined to the gut, which in turn also contain less tryptase when compared to cutaneous mast cells, and
    3.3. Loss of tryptase into the gut lumen resulting in lower concentrations reaching the systemic circulation.[51]

Opioids and mast cell activation

True type-one hypersensitivity reactions to opioids are very rare,[36] being implicated in only one percent of all assumed perioperative anaphylactic events.[61] Many of the frequently used opioids such as morphine, pethidine and codeine are histamine releasers.[61,62] The importance of this is that these drugs are capable of producing signs and symptoms that imitate true anaphylactic reactions. However, in contrast to true anaphylactic reactions these symptoms might not return or may be less severe if the agent is administered more slowly or at a lower dose.[43] Opioids in general differ in their ability to provoke mediator release from mast cells, and different mast cell populations are not equally responsive to opioids.[63,64]

Fentanyl is a potent synthetic opioid that is popular in the perioperative period due to its rapid onset and short duration of action. Even though fentanyl is roughly ten times as potent as morphine it results in little or no histamine release, as it is unable to activate mast cells. Skin reactions have been credited to direct dilatory actions on capillaries.[61]

Morphine is an opioid analgesic drug that exerts most of its effects through activation of Mu-receptors. When given rapidly or in large doses, histamine release is a frequent side effect, resulting in undesired hemodynamic changes, particularly hypotension and increased cardiac output.[65] Hypotension after the administration of large doses of morphine appears to be mediated by both the direct vasodilating actions on veins by a yet unknown mechanism and because histamine release results in venous and arteriolar dilatation decreasing systemic vascular resistance.[61]

In-vitro studies by Stellato et al. and Veien et al. showed that mast cell tryptase is released along with histamine from isolated human skin mast cells after morphine administration.[63,66] Furthermore, morphine was shown to produce dose-dependent release of mast cell mediators' tryptase and histamine from cutaneous mast cells in-vitro, without stimulating the production of prostaglandins.[63,65] Morphine appears to degranulate skin mast cells directly and non-immunologically[66] liberating histamine[36] and mast cell mediators independently of opioid receptors or opioid-specific IgE.[61,62] The precise mechanism is still uncertain. Currently, in-vivo studies evaluating the effects of large intravenous doses of morphine on systemic tryptase levels are lacking. An in-vivo study by Blunk et al. using intra-dermal microdialysis demonstrated morphine's ability to activate skin mast cells with the subsequent release of histamine and tryptase.[62] They speculated that the mechanism could be through concentration-dependent, direct activation of mast cell G proteins. Rook et al. investigated the effects of high dose pharmaceutically prepared heroin on mast cell tryptase levels in patients who were chronically treated with both heroin and methadone for heroin addiction. He concluded that following

intravenous heroin injection plasma tryptase levels increased dose-dependently (average increase was 23%). Interestingly, the percentage change in tryptase levels related to the peak morphine concentrations and not heroin concentrations.[67] A study by Fisher et al. investigated systemic mast cell tryptase levels in 350 patients with suspected anaphylactic reactions whilst undergoing anaesthesia. They concluded that mast cell tryptase is a very sensitive and useful indicator of anaphylactic reactions, although it cannot distinguish between an IgE and non-IgE mediated process.[68-71] A prospective study by van der Linden et al. investigated the relationship between the severity of insect sting induced anaphylaxis and plasma levels of mast cell mediators. They demonstrated that mast cells are activated during insect-sting anaphylaxis and that the severity of the subsequent anaphylactic episode correlated with the circulating levels of both histamine and mast cell tryptase, but not with the levels of prostaglandin (PGD_2).[72]

The rationale for using morphine rather than fentanyl during cardiac surgery is much debated. Fentanyl is a popular opioid choice in cardiac surgery due to its potency and cardiovascular stability. Fentanyl, unlike morphine, does not activate mast cells to release histamine, thus avoiding hypotension, which may follow after rapid or large dose morphine administration. However, morphine's cardioprotective, anti-inflammatory and advantageous post-operative analgesic profile makes it a favourable choice for use in patients during cardiac surgery and cardiopulmonary bypass.

The cardioprotective properties of morphine have been shown in in-vitro animal studies. Liang et al. demonstrated that the activation of functional opioid receptors on myocytes "mimics the cardioprotective effect of ischaemic preconditioning".[73] This effect was dose-dependent and blocked by naloxone. The precise mechanism of morphine's preconditioning effect on myocytes remains uncertain. Animal data suggests that it occurs through activation of the $delta_1$ opioid receptor. This direct receptor activation is thought to result in activation of mitochondrial

potassium ATP channels with a resultant increases in intracellular free radicals.[73]

Morphine possesses beneficial anti-inflammatory and immune-regulatory properties. Patients undergoing coronary artery bypass graft surgery and cardiopulmonary bypass who received morphine rather than fentanyl demonstrated reduced levels of pro-inflammatory cytokines as well as reduced expression of neutrophil adhesion molecules.[74,75] Fentanyl, on the other hand, does not appear to have the same immune regulating properties exhibited by morphine.[76]

Patients who had received morphine rather than fentanyl during elective cardiac surgery and cardiopulmonary bypass experienced less early post-operative pain, had significantly better subjective quality of recovery scores as well as less febrile episodes during the first three post-operative days.[75]

The study in article format

Title: The effect of high dose morphine compared to fentanyl infusion on serum levels of mast cell tryptase during cardiac surgery

Short title: Mast cell tryptase following high dose morphine

Abstract

Background: Morphine directly activates cutaneous mast cells in a seemingly dose-dependent manner, resulting in the release of both histamine and tryptase into the bloodstream. Tryptase is almost exclusively stored in mast cells. Elevated serum tryptase concentrations serve as an indicator of mast cell activation and have become the most frequently used laboratory investigation in anaphylaxis. Following a clinical diagnostic dilemma our study was aimed at answering whether mast cell tryptase concentration remains useful in supporting the diagnosis of anaphylaxis even after administration of high dose morphine.

Methods: We conducted a non-blinded, randomized controlled trial comparing the effects of fentanyl and high dose morphine, on serum mast cell tryptase concentrations. A power analysis was performed. Twenty adults undergoing cardiac surgery were randomly assigned to one of two opioid regimens. Both groups received a fentanyl bolus of 3 to 8 mcg/kg at induction. In the fentanyl group this was followed by a fentanyl infusion of 5 to 10 mcg/kg/hr until completion of surgery. Patients in the morphine group received morphine 1 mg/kg infused over thirty minutes. Baseline serum mast cell tryptase concentrations were determined directly prior to induction of anaesthesia and again 90 minutes after the start of the opioid infusion. The primary endpoint was statistical differences in tryptase concentrations between the morphine and fentanyl groups at the two time periods.

Results: Ten patients of similar demographics were enrolled in each group. In the fentanyl group the second, 90-minute mast cell tryptase concentration was statistically significantly (10.1%) lower (p = 0.006) than baseline. Despite the 95% confidence interval of the difference between the means (-1.06 to -0.34 mcg/L) not including zero , this was not a clinically important difference.

In the morphine group serum mast cell tryptase concentrations in the second (90 minute) sample were not statistically different from baseline values, the 95% confidence interval including zero. No between-group differences in tryptase concentration, were detected. One patient in the morphine group exhibited a clinically significant 50,4% increase in tryptase concentrations, albeit from a high baseline of 11.9 mcg/L, which in this small study constitutes a prevalence of 10% (95% CI 1.8% to 40.4.)

Conclusion: In this small pilot study, serum mast cell tryptase concentrations were unaffected by whether fentanyl or high dose morphine was administered. The null hypothesis, that there is no significant increase in serum mast cell concentrations after high dose morphine compared to fentanyl during cardiac anaesthesia and surgery, was therefore accepted. Larger studies are however needed to ensure a robust result, especially in the morphine group.

Keywords

Mast cell tryptase

High dose morphine

Anaphylaxis

Background and rationale

At the end of an otherwise uneventful hip arthroplasty conducted under spinal anaesthesia, a patient known with single vessel coronary artery disease suffered a cardiac arrest. Spontaneous circulation was restored after only 3 CPR cycles. An attending anaesthesiologist suggested administration of a large 1 mg/kg dose of morphine would facilitate overnight sedation and ventilation. This was administered over approximately 40 minutes. Towards the end of the morphine infusion, urticaria were noted on the patient's legs. Serum tryptase (34mcg/L) was significantly elevated. While anaphylaxis was suspected as a cause for the cardiac arrest, the question arose whether the morphine, particularly in such a high dose, could explain the increased tryptase levels. Despite conclusive evidence of histamine release following morphine administration,[61,65] we could not identify human research indicating the effects of high dose morphine on tryptase concentrations.

As high dose morphine is commonly used in cardiac surgery our institution, we elected to investigate tryptase levels after high dose morphine.

Methods

Hypotheses

The null hypothesis was that there is no significant increase in serum mast cell tryptase levels after high dose morphine administration in humans undergoing cardiac surgery. The alternative hypothesis was that serum mast cell tryptase levels rise significantly to levels seen during anaphylaxis after administration of high dose morphine in humans undergoing cardiac surgery.

Primary endpoints of the study

The primary endpoint was statistical and clinical (20% increase from baseline) differences in tryptase levels between the groups M and F at the two time periods.[31,58]

Ethics and consent

Ethics approval was obtained before commencement of the study (Addendum A). Informed consent was obtained before enrolment into the study.

Inclusion criteria

Inclusion criteria comprised the following:

1. Patients scheduled for elective or emergent cardiac surgery,
2. Patients with both good or poor left or right ventricular function,
3. Patients receiving aspirin and/or heparin preoperatively,
4. Patients scheduled for valve and coronary artery bypass grafting, or both,
5. Patients scheduled for either on or off pump coronary artery bypass grafting,
6. Patients with a definitive history of allergy to penicillin, although cephalosporin will also be avoided in these patients, and/or
7. Patients with an intra-aortic balloon pump already in situ.

Exclusion criteria

Exclusion criteria comprised the following:

1. Patients scheduled to undergo non-cardiac surgery,
2. Previous history of allergy to anaesthesia agents or drugs commonly used during anaesthesia and surgery, including muscle relaxants and/or opioids, particularly morphine, latex or "Elastoplast"®,
3. Patients with a diagnosis of asthma,
4. Patients with current bronchospasm from any cause, such as pulmonary oedema,
5. Patients receiving steroids, antihistamines or chromoglycate, both pre- or intra-operatively,
6. Patients who had any form of unregulated mast cell proliferation such as urticaria pigmentosa, systemic mastocytosis, or any concurrent haematological malignancy,
7. Patients with current or previous diagnoses of pheochromocytoma, paraganglioma, or carcinoid tumours,
8. Patients known with or suspected to have malignant hyperpyrexia,
9. Patients having received morphine for any reason except premedication in the previous 24 hours,
10. Patients with severe hypotension preoperatively, defined as a mean blood pressure of 60 mm Hg or less,
11. Monoclonal Mast Cell Activation Syndrome,
12. Patients where postoperative, overnight ventilation was not intended,
13. Patients for thoracotomy for lung surgery,

14. Patients with known hypothyroidism,

15. Patients with known hepatic disease severe enough to cause abnormality in bilirubin, albumin concentration or abnormal international normalized ratio.

16. Patients with renal insufficiency such that they are on dialysis or have an estimated glomerular filtration rate less than 50 ml/minute, and/or

17. Patient with a body mass index of more than 35 or less than 18 kg/m^2.

Sample size and randomization

We could identify no studies on which to base a power analysis. We therefore assumed that mean serum tryptase concentrations of 2 and 8 mcg/L with standard deviations of 2 and 8 mcg/L respectively could be expected in the control and morphine groups. Inputting these assumptions into Sigmastat® for Windows® version 2.03 indicated that studying 10 patients in each group would achieve a 80% chance of identifying a difference between groups with a 5% alpha error.

Randomization was attained by blind drawing of an envelope by the anaesthesiologist containing the data sheet and group selection.

Anaesthesia premedication

Premedication comprised diazepam 5 to 10 mg administered orally two hours before arrival in the induction room. Premedication explicitly excluded morphine or promethazine.

Conduct of anaesthesia

Except for the opioid administration, conduct of anaesthesia was left to the discretion of the attending anaesthesiologists. It was important that the conduct followed the routine, standard conduct of anaesthesia and surgery in our institution so that the results of the study are clinically applicable. The choice of drugs for maintenance and induction of anaesthesia was determined by the attending anaesthesiologist.

A fentanyl bolus of 3 to 8 mcg/kg, was used during induction of anaesthesia.

Vecuronium 0.15 mg/kg or rocuronium 1 mg/kg was administered to facilitate tracheal intubation.

After induction and tracheal intubation, the patients were randomized into one of two groups:

1. **Group M:** morphine 1 mg/kg lean body weight was administered after induction of anesthesia and completion of tracheal intubation. The total dose morphine was diluted to 50 millilitres in a syringe with normal saline and administered over 30 minutes. This practice was commonly performed in approximately one third of cases in our institution. This served as the intervention group.
2. **Group F:** Fentanyl group: administration of fentanyl as an infusion at 5 to 10 mcg per kg per hour until the completion of anaesthesia. This served as the control group.

Blood sampling and processing

Five millilitre blood samples were obtained from the intra-arterial cannula at the following times:

1. Before induction of anaesthesia, after the placement of the intra-arterial catheter but prior to administration of antibiotics.

2. Sixty minutes after completion of the morphine infusion. This was to be before starting cannulation for initiation of cardiopulmonary bypass.

Samples were processed in a particular way. Blood samples were stored in 5ml EDTA gel tubes. These specimens were stored at 2 to 8 degrees centigrade in our hospital. Within 3 days, specimens were transferred to the UCT Lung Institute in Mowbray, Cape Town, South Africa. At the Lung Institute, samples were spun down at 4000 revolutions per minute; the serum was decanted and then frozen at -20 degrees Celsius. Once a sufficient number of samples were available, a batch analysis for tryptase was performed, a more cost effective method. 40 samples were tested at a price of R192 each, compared to R900 normally charged for a single sample at the National Health Laboratory Service in Cape Town, South Africa.

Total serum tryptase levels were measured using the ImmunoCAP® method (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden). Three monthly external tryptase quality controls, specific to this method, were performed. A particular trained technician, Ms. Bartha Fenemore, was responsible for all tryptase analyses.

We derived serum tryptase reference values from a study in 126 healthy persons aged between 12 to 61 years indicated a geometric mean of 3,8 mcg/L and an upper 95^{th} percentile of 11,4 mcg/L. This study is reported in the package insert and website of the only commercially

available test for measuring Tryptase concentrations, the ImmunoCAP® method. However, it is not clear whether this data has been peer reviewed and/or published as a paper.

The data was inputted into an Excel® spread sheet and all data points were checked before analysis.

Results

Medcalc® version 15 was used to perform statistical analysis. Data was tested for normality of distribution (Kolmogorov Smirnov test; $p < 0.05$) and equality of variance (Levene Median test; $p < 0.05$). The Kolmogorov-Smirnov test revealed that all data was normally distributed. Normally distributed, parametric data, which exhibited equal variance, was analysed with the appropriate t-test. The one instance of between-group data (second sample) with unequal variances was analysed using nonparametric (Man Whitney) tests. The strength of association for data that was both normally distributed and also exhibited a constant variance was measured using the Pearson product moment correlation. Otherwise, Spearman rank order correlation was be used to measure the strength of associations.

Ten patients per group were enrolled in the study. There was no missing data. Demographics (age, gender, height, weight and surgical procedure (Table 2) did not differ between groups.

Table 2.

	Fentanyl			Morphine			p	95% CI of difference between mea
	Mean	**Standard Deviation**	**95% Confidence intervals**	**Mean**	**Standard deviation**	**95% confidence interval**		
Age	60	8.4	53 -67	57	14.6	44-70	0.6444	-13,69 to 8,69
Weight	77	20	59- 87	71	13	60-80	0.4225	-21,89 to 9,59
Height	151	53	156-174	169	13	160-178	0.937	-0.10 to 0.10
Gender	Female=1 Male=0			Female=3 Male=7			0,513	
ASA	ASA 2=2 ASA 3 =8			ASA 2 = 3 ASA 3 = 7			1	
Surgery	CABG x10			CABG x 10			0,823	
Allergy history	None			One penicillin			1	

Study participants demographic data

Results are presented in Table 3 and 4 as means and standard deviation, and as medians and interquartile ranges, respectively. The 95% confidence intervals are presented for both means and medians. The 95% confidence intervals for non-parametric data were determined using Confidence Interval Analysis® Software, version 2.2.0 (BMJ Books, London, UK).

Table 3.

Opioid	Tryptase concentration sample 1	95% confidence interval for the median	Tryptase concentration sample 2	95% confidence interval for the median	P (Wilcoxon signed ranks)	95% confidence interval of Difference
Fentanyl	5.40	4.08 to 6.99	4.85	3.33 to 6.14	0.006	-1.06 to -0.34
Morphine	4.92	3.25 to 9.67	4.47	3.33 to 10.02	0.56	-0.79 to 2.29

Data presented as medians and 95% confidence intervals of the median. Concentrations measured in mcg/L.

Table 4.

Opioid	Tryptase concentration sample 1 Mean ±Standard deviation	95% CI mean	Tryptase concentration sample 2	95% confidence interval for the mean	Two tailed probability	95% confidence interval of difference
Fentanyl	5.6	4.2 to 7.0	4.9	3.7 to 6.1	P= 0.002	-1.1 to -0.3
Morphine	6.1	3.3-9.0	6.3	2.6-10.0	P= 0.7	-1.3-1.76

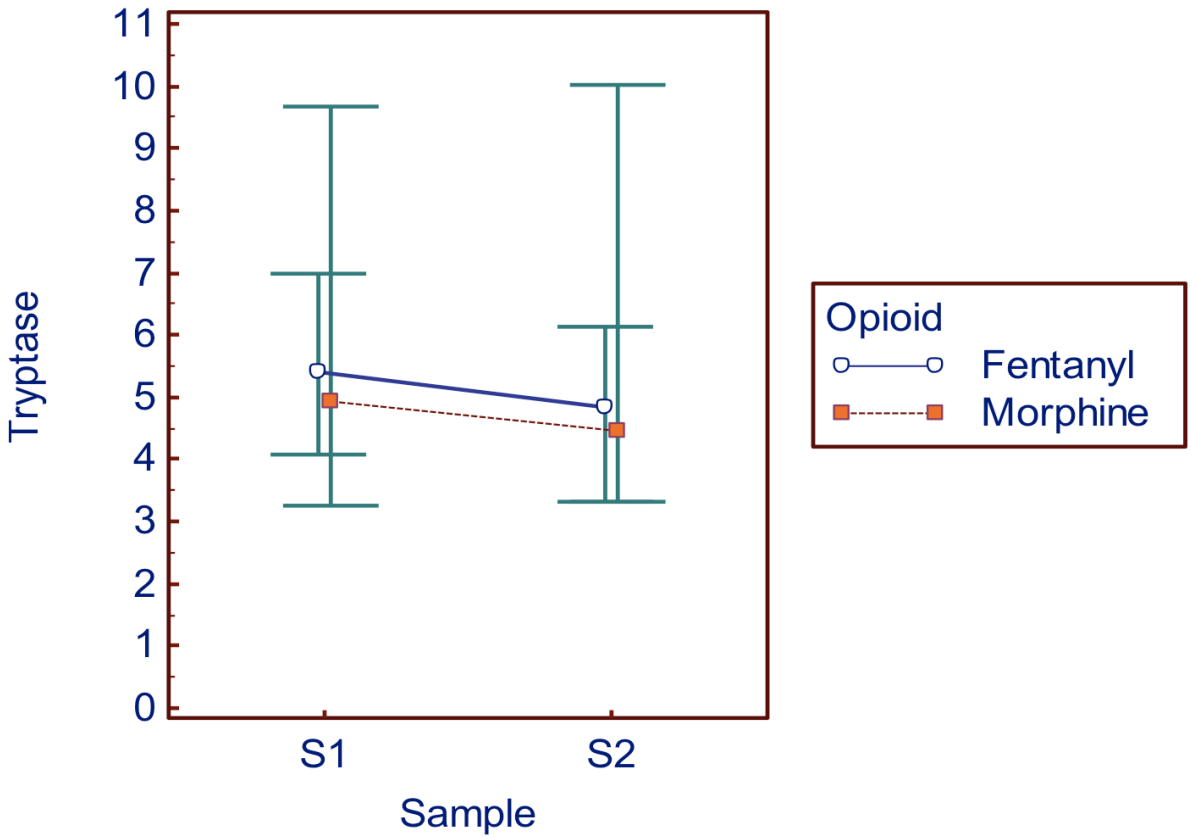
Data presented as means ± standard deviation and 95% confidence intervals of the mean. Concentrations reported in mcg/L.

In the fentanyl group, baseline tryptase concentrations were all within the reference range. The second, 90-minute tryptase concentration was (10.1%) statistically significantly lower than baseline, the 95% confidence interval of the difference between the means not including zero. The low p-values indicated only a small chance of incorrectly making such a conclusion. This decrease in tryptase concentration was not clinically significant (fig 2).

In the morphine group, baseline tryptase concentrations were within the reference range. Serum tryptase concentrations in the second (90 minute) sample were not statistically different from baseline values, the 95% confidence interval including zero. No clinical difference between the initial and follow-up tryptase concentrations was observed (fig 2).

No between-group differences in tryptase concentration were detected.

Figure 2.



Graph of median values with error bars displaying the 95% confidence intervals.

Discussion

We conducted a non-blinded, randomized controlled trial comparing the effects of fentanyl, or fentanyl followed by high dose morphine, on total serum tryptase concentrations. Fentanyl administration alone resulted in a statistically significant, but clinically insignificant 10,1% decrease in total serum tryptase. (Fentanyl followed by) high dose morphine administration did not alter the follow up tryptase concentrations. In summary, total baseline tryptase was unaffected by either fentanyl or high dose morphine administration. The null hypothesis, that there is no significant increase in serum mast cell tryptase concentrations after high dose morphine compared to fentanyl during cardiac anaesthesia and surgery, was therefore accepted.

Opioids and mast cell activation

Mast cell activation is best known as the ultra-rapid degranulation as seen in anaphylaxis.[17,19] However, slower, selective secretion of granular content and/or selective chemokine synthesis alone can occur.[77] Morphine activates mast cells non-immunologically, independent of both opioid receptors and opioid-specific IgE,[16,61,62,66] without stimulating prostaglandin production.[64,65] Morphine possibly initiates mast cell degranulation via concentration-dependent, direct activation of mast cell surface G proteins.

Mast cells occur in many tissues, with mast cells populations in different locations varying with respect to their granular contents, susceptibility to activation, and post-activation biological responses.[2,21,78] This mast cell inhomogeneity is attributed to differences in mast cell surface receptors as well as downstream signalling pathways. The detailed biology of the inhomogeneity is not yet well understood. Indeed, individual mast cell populations react differently to opioids. In-vitro studies by Stellato et al, Veien et al, and Blunk et al. demonstrated morphine predominantly activates the cutaneous mast cell population.[61,64,66]

Opioids vary as to their ability to activate mast cells. Authoritative anaesthesia and pharmacology textbooks state that intravenous administration of certain commonly used opioids, including morphine, pethidine, and/or codeine, initiate mast cell activation. [79,80] This is followed by increases in plasma histamine concentrations. The higher the opioid dose, the greater the extent of mast cell activation, histamine release and systemic hypotension. However, fentanyl and its derivatives do not activate mast cells or initiate histamine release.[61,62,64,65,79,81] In this light, it was surprising that high dose morphine did not result in a measurable increase in serum tryptase concentrations.

No change in tryptase with morphine

Mast cell tryptase concentrations did not differ statistically or clinically from baseline following morphine administration. In addition, no post- opioid administration, between-group tryptase concentration differences were observed. As far as we can determine, this has been not been described before. This is an interesting finding as we expected that morphine would cause a dose-dependent rise in tryptase concentrations. Indeed, Rook et al. observed that the increase in plasma tryptase following intravenous heroin was related to the peak morphine concentrations and not heroin concentrations.[67]

It is interesting to speculate why the expected rise in tryptase concentrations did not occur. As histamine concentrations increase after morphine administration,[61,64,82] we cannot conclude that mast cell degranulation with release of granular tryptase does not occur. Morphine selectively activates (cutaneous) mast cell populations and particular mast cell pathways.[64] It may be that the extent of cutaneous mast cell activation may not be sufficient to elevate systemic levels.

We also questioned whether we missed the peak rise in tryptase concentrations. The second sample, presumably indicating peak tryptase concentrations, was collected 90 minutes after commencement of the morphine infusion. The rationale for sampling at 90 minutes was that Schwartz et al observed peak beta tryptase levels to occur between 1 to 2 hours after wasp sting venom induced anaphylaxis.[48] Thus, taking the second sample at 90 minutes would presumably reflect post morphine, peak tryptase concentrations. It may be that this was not the correct model on which to base the effects of morphine. It may have been that the slow rate of morphine infusion with gradual mast cell activation resulted in a smaller peak tryptase concentration occurring later than 90 minutes after start of the infusion. Thereby sampling at 90 minutes missed peak tryptase levels. On the other hand, the dose of morphine administered was large, and the period over which it was administered was relatively short. We would have expected a small rise in tryptase if it did occur. However, we could not delay tryptase sample collection any further as this would have been during initiation of, or even during, cardiopulmonary bypass. Nonetheless, should anaphylaxis be suspected and tryptase levels were raised, can conclude that this rise in tryptase was unlikely to be due to recent (within 60 to 90 minute) large dose morphine administration.

Administration of larger volumes of intravenous fluid in response to hypotension in the morphine group could have resulted in falsely low peak tryptase concentrations. We did not formally assess hypotension or other hemodynamic alterations during opioid administration. Furthermore, we did not investigate fluid administration between the first and second sample neither the fluid administration nor hypotension in either group. These are weakness of this study. We also did not document other clinical signs associated with mast cell degranulation such as evidence of bronchospasm, or skin manifestations. All these details will require recording in future, similar studies.

One patient exhibited a clinically significant increase in tryptase concentrations, which in this small study constitutes a prevalence of 10% (95% CI 1.8% to 40.4) (Figure 2). In this case, baseline and post morphine tryptase were 11,9 and 17,9 mcg/L respectively. This represents a baseline tryptase higher than what is regarded as normal and the 50.4 % rise is out of keeping with the other results in the morphine group. This patient could have had an underlying, undiagnosed mast cell disorder.

The question is whether this single outlier invalidates the study conclusions? The absence of similar, previous research meant that we had to estimate values on which to base our power analysis. The results of our small pilot study did achieve a statistical and clinical significance. However, the outlier suggests more subjects need to be studied to ensure a robust result, particularly in the morphine group. The value of this study is that it provides valuable, hitherto unavailable data on which a larger study can be based. Using our data, a sample power calculation using a paired t-test (PASS sample size software, NCSS, LLC) concluded that a sample size of 667 would achieves 80% power to detect a difference of -0.2 between the null hypotheses mean of 6.1 and the alternative hypothesis mean of 6.3 IU/ml with a known standard deviation of 2.1 and with a significance level (alpha) of 0.05." Assuming a 5% to 15%, prevalence of tryptase exceeding 11.4 mcg/L, it would require a sample size of 134 in order to narrow down the 95% CI.

Use of tryptase in diagnosis of anaphylaxis

Tryptase is almost exclusive to mast cells, with serum mast cell tryptase concentrations being the most frequently used laboratory test in suspected anaphylaxis.[58] Elevated serum tryptase levels are an indicator of mast cell activation.[53] The clinical scenario we were confronted with was whether elevated tryptase levels were indeed due to allergy or if it could be attributed to morphine. Our study was aimed at answering whether mast cell tryptase levels remain useful in supporting the diagnosis of anaphylaxis after administration of high dose morphine. The ability

to confirm anaphylaxis will initiate the post-operative process of identification of the offending allergen and future avoidance of the probable allergens.

Baseline serum tryptase concentrations result from secretion of "immature" pro-tryptases by resting mast cells, the 95% CI upper limit being less than 11.4 mcg/L.[26] Mast cell granules only store "mature" beta tryptase, which is released only after mast cell activation. Mature beta tryptase should not normally be present in serum. Its presence denoting mast cell degranulation.[26] Mature beta tryptase concentrations peak one hour after a serious allergic reaction, elevated for up to six hours, and achieve baseline levels only after 14 hours.[26] An increase in total tryptase levels of 20% or more from the baseline is suggestive of mast cell activation,[26,58] and a ratio of total to mature beta tryptase of 10 or below is indicative of anaphylaxis.[26] Tryptase concentrations are much more likely to be raised when anaphylaxis is triggered by a parentally administered allergen and when the reaction is severe (i.e. resulting in hypotension).[24] This is probably due to delivery of large amounts of allergen directly to mast cells resulting in rapid and extensive mast cell degranulation.[26] The current commercially available serum tryptase measurement methods available in South Africa indiscriminately measure both the immature and mature tryptase levels.

Rationale for using morphine during cardiac surgery.

Morphine is even cheaper to acquire than fentanyl, a consideration in our cost-conscious environment. One risk is that injudicious use, particularly of excessive dosages of morphine may delay awakening and tracheal extubation, and be associated with additional costs.

Fentanyl remains a popular opioid in cardiac surgery due to its potency and cardiovascular stability. Unlike morphine, fentanyl's onset is relatively rapid. Fentanyl does not activate mast cells,[64,65] and is reported to produce less hypotension than morphine. [81,82] It is thus very suitable for use during induction of anaesthesia for cardiac surgery.

Morphine mimics the cardioprotective effect of ischemic pre-conditioning.[73,74] Furthermore, morphine,[74,75] unlike fentanyl[76] possesses beneficial anti-inflammatory and immune-regulatory properties. Morphine appears to lessen the inflammatory response after cardio-pulmonary bypass through binding to the mu -3 morphine selective receptor, which fentanyl does not bind. A study by Murphy et al. showed significantly lower interleukin 6 levels after cardio-pulmonary bypass in patients receiving morphine rather than fentanyl during cardiac surgery.[74]

Patients who received morphine rather than fentanyl during elective cardiac surgery and cardiopulmonary bypass experienced less early post-operative pain, had significantly better subjective quality of recovery scores as well as less febrile episodes during the first three post-operative days.[75]

Remifentanyl is an ultra-fast-acting opioid often used during cardiac surgery. Its unique pharmacokinetic and pharmacodynamic profile allows for rapid onset of intense analgesia as well as fast and predictable recovery on cessation of the infusion. The concern with using remifentanyl is that hyperalgesia and opioid tolerance increases post-operative pain during major abdominal surgery.[83] These finding were confirmed in a recent systematic review and meta-analysis on randomized, controlled studies by Fletcher et al. They concluded that the use of high doses of remifentanyl intra-operatively significantly increased pain perception during the first 24 hours after surgery, with moderate increases in the amount of morphine required during that period.[84] Another serious concern with the use of remifentanyl during cardiac surgery is that it may be associated with chronic pain after sternotomy,[85] prospective studies are currently underway to investigate whether remifentanyl does indeed increase the incidence of chronic pain after cardiac surgery.

The decrease in tryptase with fentanyl

The decline of mast cell tryptase concentrations in the fentanyl group was an interesting, unexpected observation. The small 10.1% decrease was statistically significant with narrow confidence intervals confidence and low p-values. Despite being clinically unimportant, it is tempting to comment on this result. This finding has not been described before as far as we can determine. The fentanyl group decrease in serum mast cell tryptase concentrations may possibly be a result of haemodilution secondary to fluid loading, although this would rather be

expected in the morphine group. We cannot validate these speculations, as we did not quantify hypotension or assess fluid administration. The one convincing conclusion is that a rise in tryptase concentrations following fentanyl administration is not a physiologically expected result. Unfortunately, the low numbers enrolled with low statistical power means that the statistically significant result in the fentanyl group is less likely to reflect a true difference.

Summary and conclusions

Mean serum tryptase concentrations did not increase 60 minutes after infusion of high dose, 1 mg/kg, morphine administered over 30 minutes. The one outlier, with a baseline greater than normal and consequent 50.4% rise in tryptase levels, complicates the results. At the time of writing, we could identify no previous in-vivo studies evaluating the effects of large intravenous doses of morphine on systemic tryptase concentrations. In effect this is a pilot study. The value of the study is that it provides valuable, hitherto unavailable data on which a larger study can be based. A larger study will be needed to confirm the morphine group results. Fentanyl administration was followed by a 10.1% statistically significant, but clinically insignificant "decrease" in serum tryptase. This study indicates that a rise in serum tryptases unlikely to be due to fentanyl administration per-se.

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